Development of West Nile virus Testing and Donor screening as a Model for Screening Bioterrorist Agents

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Office of Blood Research and review Counterterrorism strategic plan

- Facilitate the development and availability of safe and effective medical products to prevent and treat the health consequences of terrorist event
- Protect the integrity of the blood supply and other biological products
- Provide accurate and timely information regarding medical products used to prevent or treat consequences of terrorist acts
- Enhance emergency preparedness and response capability

Office of Blood Research and review Counterterrorism strategic plan

- **Protect the integrity of the blood supply and other biological products**
  - Devise and implement donor screening methods and tests to assure that affected individuals do not spread agent
    - Investigate testing methodologies for screening donors
    - Develop policies for screening and standards for lot release and validation assays
    - Assess studies characterizing agent pathogenesis
    - Communicate with manufacturers for development of screening assays
  - Develop a program to evaluate approved and unapproved methods for removal and inactivation of possible bioterrorist infectious agents
    - Pathogen removal and inactivation
    - Develop antiviral compounds

Office of Blood Research and review Counterterrorism strategic plan

- Investigate testing methodologies for screening donors
  - Develop Real Time PCR and hybridization to a microarray of DNA oligonucleotides to detect bioterror agent (BT) nucleic acids in blood and blood products in a multiplex format
    - Accomplishments:
      - Optimized Real time PCR amplification for 3 category A agents
      - Achieved simultaneous detection of cells from the 3 BT agents in blood samples using microarray platform

Development of WNV donor screening test as a model for BT agent screening in blood and blood products

Progress in Donor Screening for West Nile virus

- FDA's actions to date regarding donor testing
- WNV test development including the lot release and validation panel development
- WNV testing of blood donors using investigational tests

West Nile Virus and Blood Safety: FDA's Actions to Date

- Alert notices posted on FDA's website:
  - August 17, 2002: Vigilance in excluding symptomatic donors urged prior to any actual report of transmission
  - October 3, 2002: FDA states its interest in facilitating development of donor screening & supplemental tests
- Congressional hearings or briefings on September 10 & 24, October 3, 2002, and June 6, 2003
- FDA is working closely with the test kit manufacturers to expedite test development and implementation (Sept. 2002 industry meeting, Nov 2002 FDA scientific workshop, BPAC update in 12/02, BPAC discussion 3/03)
- FDA issued a guidance on "Recommendation for the assessment of donor suitability and blood and blood product safety in cases of known or suspected West Nile virus infection", Oct. 25, 2002
- FDA issued a revised guidance on "Revised recommendation for the assessment of donor suitability and blood and blood product safety in cases of known or suspected West Nile virus infection" May 1, 2003

West Nile Virus and Blood Safety: FDA's Actions to Date

- FDA has approved:
  - GenProbe (16 samples/pool) phase 1 IND for repository testing on March 21, 2003
    - phase 2 IND for prospective WNV NAT testing on May 27, 2003
  - American Red Cross IND for WNV NAT testing (GenProbe test 16 samples/pool) on May 27, 2003
  - Roche IND for WNV NAT testing (6 samples/pool) on May 22, 2003.
- Investigational WNV NAT testing has started since mid June 2003 using pooled or individual samples.
- FDA is participating in weekly meetings with the task force established by blood banking community, which includes CDC and NIH to coordinate the epidemiological data on WNV infection and to monitor the out come of testing.

Background Information

- WNV is an enveloped single stranded RNA virus
- WNV is a mosquito-borne flavivirus
  - Primarily infects birds
  - Occasionally infects humans and other animals
- About 80% of human infection is asymptomatic, and 20% develop mild febrile illness (flu-like illness)
- Approximately 1 in 150 infections results in meningitis or encephalitis
  - Advanced age is by far the most significant risk factor for severe neurologic disease
- Viremic period can occur up to 2 weeks prior to symptoms and last up to a month from the initiation of the infection

Background Information……

- The 2002 US outbreak of WNV resulted in the identification of other modes of transmission including:
  - Blood transmission (RBCs, plasma and platelets), Transplantation, Breast-feeding, Transplacental and Occupational by percutaneous injury
- The magnitude of the risk of WNV from transfusion is unknown.
- Virus titer in blood is low compared to other transmissible viruses (~1-5x10^3 copies/ml) and the viremia is transient.
  - Viremia in encephalitis patients can be as high as 2.5x10^6 copies/ml
- Viremia resolves rapidly after seroconversion to IgM
- IgM can persist for a long time in some cases up to 2 years
- No chronic stage of WNV infection has been reported

Status of WNV pathogenicity and epidemiology in the US in year 2002

- In year 2002 total number of WNV cases reported were 4156 out of which 284 deaths and 2942 cases of WNME
- 44 states including DC are endemic for WNV
- The average risk of WNV by transfusion in 2002 was 0.4 per 10,000 donations nation wide with a maximum risk 10.46/10,000 donations in Michigan
- During Aug 28, 2002- June, 2003, 61 possible Transfusion-Transmitted cases reported (Retrospective testing of 2002 epidemic)
    - 23 are confirmed from 16 blood donations
    - 19 are not transfusion related,
    - 19 inconclusive due to incomplete donor follow-up
    - 6 deaths- WNV could not be established as the cause in most cases

FDA's Research Activities on WNV

- Panel development
  - To monitor sensitivity of assays to detect viral nucleic acids and antibodies
- Isolation and characterization of WNV strains from human samples obtained during the 2002 and 2003 epidemics
  - Genetic variation of viral strains
  - Detection by currently available WNV assays
- Natural history studies to evaluate infectiousness in blood to better understand risk and define unit/donor management strategies

Analytical sensitivity of WNV assays

- FDA's current standard for licensure WNV NAT assays is 100 copies/ml for the individual donation
- WNV panel designed to monitor this sensitivity limit
- Standard may be revised based on infectivity data or sensitivity improvements

FDA NAT Panels

- FDA NY99 and FDA-Hu2002 isolates characterized by genetic sequencing
- Viral infectivity determination
- RNA concentration measurements
- Final panel specifications are being established through collaborative studies
  - The prototype panel consists of two isolates
  - Viral concentration ranges between 1000-5 copies/ml

Viral Titer Determination Copies/mL

Isolate (dilution)	Average of multiple testing performed by each laboratory*					Final Copies/ml
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	
FDA-Hu2002 (10^{-1})	10^9	10^9	10^9	10^9	10^9	10^{10}
FDA-Hu2002 (10^{-4})	10^6	ND	10^6	10^6	ND	10^{10}
FDA-Hu2002 (10^{-7})	10^3	ND	10^3	10^3	10^3	10^{10}
NY99 (10^{-1})	10^9	10^9	10^9	10^9	10^9	10^{10}
NY99 (10^{-4})	10^6	ND	10^6	10^6	ND	10^{10}
NY99 (10^{-7})	10^3	ND	10^3	10^3	10^3	10^{10}

Correlation between Copies/mL and PFU/mL

Sample	Copies/ml average (FDA & NYSDOH)	PFU/ml
FDA-Hu2002	10^{10}	10^{7}
NY99	10^{10}	10^{8}

Correlation between Copy/mL and PFU/mL

Sample	Av. copy	PFU
HuWNV	10^{10}	10^{7}/mL
HuWNV 60^{o}C/2 hr	10^{7}	0
NY99	10^{10}	10^{8}/mL
NY99 60^{o}C/2 hr	$10^{6.5}$	0

Status of WNV pathogenicity and epidemiology in the US in year 2003

- During 2003 total number of WNV human cases reported so far are ~5000 out of which 88 deaths, of the total infections ~29% cases of WNME and ~ 60% cases of WNV fever
- 44 states including Washington D.C. are endemic for WNV
- Putative WNV transfusion related cases are being analyzed
  - Confirmation of NAT and IgM reactivity
  - Donor and recipient F/U
- CDC reported two confirmed cases of WNV transmission through transfusion

West Nile Virus and Blood Safety: Current Status

- Starting July 1, 2003 blood donor screening under IND in place covering all geographic regions of the US
- **Several confirmed asymptomatic infection interdicted**
  - ~ >1000 units of WNV infected blood detected from ~ $3x10^6$ donations screened
  - Units would otherwise have been transfused
- MP-NAT testing has effectively removed >75% of infected blood donations from entering the blood supply for transfusion

West Nile Virus and Blood Safety: ID-NAT study

- Potential for transmission of WNV through minipool (MP) NAT negative blood and blood components. Because of the low level of viremia in some patients and window periods of detection before and after seroconversion.
- A limited retrospective evaluation of MP-NAT negative units from 2003 epidemic from four high incidence regions (~1/250 + rate using MP) and retrospective studies on samples collected during 2002 epidemic were done using ID-NAT GenProbe test.
  - Samples with low level of viremia may be missed in minipool testing
  - Putative WNV transfusion related cases are being analyzed
    - Confirmation of NAT and IgM reactivity
    - Donor and recipient F/U

West Nile Virus and Blood Safety: ID-NAT study

- **Goals of the ongoing ID-NAT study are:**
  - To perform ID-NAT prospective and retrospective testing in high incidence areas, F/U and determine infectivity of such units
  - Compare testing of the ID-NAT positive samples between the two test kit manufacturers
  - Perform an infectivity study in various animal models including non-human primates and using the MP-NAT (-), ID-NAT (+) units

West Nile Virus and Blood Safety: ID-NAT testing

- Pending data on the infectivity of MP NAT-/ID NAT reactive units
  - ID-NAT is being performed prospectively in high incidence areas based on the capacity for additional testing and the frequency of MP-NAT + units collected in the region.
  - Frozen plasma is being withdrawn in areas with high incidence of WNV based on MP-NAT.

West Nile Virus and Blood Safety: Summary

- Blood donor screening for WNV using investigational MP-NAT was achieved in a record time (~9 months) since FDA stated its interest in the development of donor screening test
- Because of the implementation of MP-NAT >75% of infected blood donations have been interdicted
- In addition, in limited setting ID-NAT is being performed in high incidence areas
- Studies are underway to determine the infectivity of low level viremic donations [MP-NAT (-), ID-NAT (+)]
- This has been possible due to close cooperation between public health agencies, blood establishments and the test kit manufacturers

Acknowledgements

- Task force which consists of public health agencies (FDA, CDC. NIH, DOD) and blood establishments for weekly updates and monitoring the progress of WNV epidemic and testing

- Test kit manufacturers for development of investigational tests in a timely manner

- FDA staff for interactive review and formulating policies

- Blood establishments for timely implementation of WNV testing